sIL2R ratio as early marker for response in hairy cell leukemia and the prognostic relevance of IL28B genotype to interferon- therapy

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Abstract: Interferon- (IFN) was the first effective drug therapy for hairy cell leukemia (HCL). Nowadays, it is used as an alternative treatment in selected patients. Due to unlimited treatment time, monitoring and early prediction of response are important. Moreover, IFN is used in the therapy of chronic hepatitis C, where a single nucleotide polymorphism of interleukin-28B gene (IL28B) correlates with therapy response. The role of this polymorphism in therapy response of IFN -treated patients with HCL is unknown. Thirty-seven HCL patients treated between 1978 and 2014 were included in this study. Treatment strategy and response parameters (blood cell counts, soluble interleukin-2 receptor (sIL2R), and bone marrow examination) have been assessed. Relative decrease of sIL2R was correlated with outcome parameters. Response parameters of IFN -treated patients were correlated with IL28B polymorphism. Twenty-one patients were analyzed for the correlation of sIL2R ratio and outcome. After 1 and 3 months of therapy (IFN or cladribine (CDA)), the median sIL2R level showed a relative decrease of 79 and 91%. These decreases significantly correlate with time to complete remission (CR, p = 0.029 and p = 0.018). Correlation analyses of IL28B genotype with outcome parameters are not significant. Six patients (16%) were diagnosed with secondary malignancies, and one death was registered (median follow-up time 14 years). IFN is a safe, effective, and well-tolerated long-term treatment in HCL. Relative decreases of sIL2R levels correlate with time to CR and are useful as early predictor for response. There is no significant correlation between IL28B polymorphism and treatment response to IFN . Graphical abstract.

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TITLE:

sIL2R ratio as early marker for response in hairy cell leukemia and the prognostic relevance of *IL28B* genotype to Interferon-α therapy

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Running title: Relevance of sILR and IL28B genotype in HCL

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ABSTRACT

Background: Interferon-α (IFNα) was the first effective drug therapy for hairy cell leukemia (HCL). Nowadays, it is used as an alternative treatment in selected patients. Due to unlimited treatment time, monitoring and early prediction of response are important. Moreover, IFNα is used in the therapy of chronic hepatitis C, where a single nucleotide polymorphism of Interleukin-28B gene (*IL28B*) correlates with therapy response. The role of this polymorphism in therapy response of IFNα-treated patients with HCL is unknown.

Methods: 37 HCL patients treated between 1978 and 2014 were included in this study. Treatment strategy and response parameters (blood cell counts, soluble Interleukin-2 receptor (sIL2R) and bone marrow examination) have been assessed. Relative decrease of sIL2R was correlated with outcome parameters. Response parameters of IFNα-treated patients were correlated with *IL28B* polymorphism.

Results: 21 patients were analyzed for the correlation of sIL2R ratio and outcome. After 1 and 3 months of therapy (IFNα or Cladribine (CDA)), the median sIL2R level showed a relative decrease of 79% and 91%. These decreases significantly correlate with time to complete remission (CR, p=0.029 and p=0.018). Correlation analyses of *IL28B* genotype with outcome parameters are not significant. Six patients (16%) were diagnosed with secondary malignancies and one death was registered (median follow-up time: 14 years).

Conclusions: IFNα is a safe, effective and well-tolerated long-term treatment in HCL. Relative decreases of sIL2R levels correlate with time to CR and are useful as early predictor for response. There is no significant correlation between *IL28B* polymorphism and treatment response to IFNα.

Keywords

Hairy cell leukemia, IL28B polymorphism, Interferon-alpha, soluble Interleukin-2 Receptor, Cladribine

INTRODUCTION

Hairy cell leukemia is a hematopoietic neoplasm of mature B-lymphocytes [1]. The first comprehensive publication on HCL was published in 1958 by Bouroncle et al. calling the disease "leukemic reticuloendotheliosis" [2]. An incidence rate of 0.3 HCL cases per 100'000 persons is estimated [3]. Male patients are 3 to 4 times more often affected than female. The median age at diagnosis varies from 55 to 65 years [3, 4]. Symptoms are not specific, but mainly related to cytopenia and splenomegaly. Fatigue, infection, hemorrhagic diathesis as well as abdominal discomfort are seen in up to 51% of cases [5]. Based on the typical morphology, the phosphatase with tartrate stain and characteristic flow cytometry showing expression of CD11c, CD25 and CD 103 [6], the diagnosis can be made with high accuracy. Nowadays, the *BRAF* V600E mutation is an additional helpful diagnostic test with high specificity [7]. The course of illness is mainly indolent. With the introduction of drug therapies (IFNα 1984, purine nucleoside analogues (PNA) 1990, Rituximab 1999) a significant increase in survival was observed. The most impressive improvement of survival is described after 1984 with the introduction of IFNα as the first specific therapy for HCL [8]. IFNα induces a rapid normalization of the peripheral blood counts resulting in a significant lower rate of infections than PNA [9]. In addition, long-term therapy with IFNα is often well-tolerated with durable disease control even on very low doses of treatment (Benz et al., 2009). However, once IFNα is completely stopped, disease recurrence is observed invariably. Nowadays, the recommended first line treatment are the two purine nucleoside analogues (PNA) Cladribine and Pentostatin, because of their high rates of CR (76-95%) and limited treatment time. Nonetheless, IFNα is still an important alternative therapy for selected patients. Beside patients who failed PNA therapy, it can be used as treatment induction in those with high risk for infections due to very low granulocytes [10, 11]. Novel treatment options as BRAF inhibitor vemurafenib and moxetumomab pasudotox, an anti-CD22 recombinant immunotoxin show promising results in early trial phase. However, they are not established in the standard treatment [12, 13].

Until recently, IFNα together with Ribavirin were recommended as first line therapy for hepatitis C virus infection. This combination induces a sustained viral response (SVR) in one half of the patients of European ancestry [14, 15]. One explanation for this significant difference was found by Ge et. al in a genome-wide association study of more than 1'600 individuals. They found a single nucleotide polymorphism (SNP) of the *IL28B* gene (chromosome 19, rs12979860), which was associated with a significantly greater rate of SVR as well as a twofold higher treatment response for genotype "CC" [16]. McCarthy et al. validated these findings in 1'021 hepatitis C patients and concluded that the SNP-marker rs12979860 is a significant predictor for treatment response to IFNα in patients with chronic hepatitis C.

In this study, we analyzed our HCL cohort with a special focus on the IFNα treated patients in regards to the impact of *IL28B* polymorphism and response to IFNα. Due to the important role of long-term follow-up in HCL patients, a newly elaborated sIL2 ratio was correlated to established outcome parameters.

METHODS

The diagnosis of all HCL patients was based on clinical presentation, peripheral blood counts and examination of bone marrow as well as flow cytometry and imaging of the spleen (ultrasound or computer tomography). From 2012 on, *BRAF* V600E mutation was as well taken into account. HCL patients at the University hospital of Zurich and the Kantonsspital Münsterlingen in Switzerland were consecutively informed about the possibility of participating in this retrospective study. Demographics and treatment specific data were collected from the electronic patient files of the two hospitals. To monitor treatment response, sIL2R was regularly measured (Cellfree® Human sIL-2R ELISA – Kit. Thermo Scientific) since the correlation between the amount of HCL cells and sIL2R value is well-established [17, 18]. In addition, sIL2R has been shown to be very useful in IFNα treated patients [19]. However, absolute sIL2R values of different patient are not comparable. We therefore established a ratio of the initial and follow-up value. To validate the usefulness of this ratio, we correlated the value to established prognostic parameters like CR.

EDTA blood samples were collected for *IL28B* genotyping. Samples were anonymized. Genomic DNA was isolated using the MagNaPure Compact instrument (Roche Diagnostics) at the laboratory of the Institute for Clinical Chemistry of the University Hospital Zurich. The *IL28B* single nucleotide polymorphism (rs 12979860) was determined by PCR followed by melting curve analysis (LightMix Kit IL-28B, Tib Molbio, Germany) on a LightCycler 2.0 instrument.

The correlation of genotypes and therapy response was studied with a special focus on the patients treated with IFNα. The following response criteria were used: Complete remission was defined as hemoglobin concentration of more than 120 g/l, absolute granulocyte count more than 1.5 G/l, platelet count of more than 100 G/l and absence of hairy cells in the peripheral blood or, if provided, bone marrow biopsy [4]. A primary resistance was diagnosed, if no normalization of peripheral blood counts was achieved after 3 months of therapy. Progressive cytopenia with proven hairy cells after CR was counted as relapse. These were, in general, verified by examination of bone marrow.

Statistical analyses were made with IBM® SPSS® version 21.0. Continuous data were tested for normality and presented as median and interquartile range (IQR). Non-parametric (Mann-Whitney) and parametric tests (t-test) were applied for group comparisons (e.g. CC genotype vs. non-CC genotype) as appropriate. Correlations of sIL2R ratio were evaluated using spearman's correlation coefficient rho. Linear regression was used to test the association between outcome parameters and genotypes. A two-sided $p<0.05$ was considered significant.

The local ethics committee approved this study (KEK ZH NR 2010-0237) and each participant signed a written informed consent and all procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008 [20].

PATIENTS CHARACTERISTICS

Thirty-seven patients could be included and analyzed. Patients were diagnosed for HCL between 1978 and end-2014. The vast majority of patients (26) were treated with IFNα, 24 of whom had first-line and 2 second-line therapy. The baseline characteristics are shown in table 1. The median follow-up time was 14 years (IQR 7-23y). Median age at diagnosis was 45 years (IQR 40-52y). Males were predominant (29; 78%, sex-rate ratio 3.6:1). At diagnosis, 10 patients (30%) presented with infections, in 11 patients (33%) fatigue was the main symptom and in 8 patients (24%) the diagnosis was made during a routine check. Only 4 patients (12%) complained about abdominal pain, even though 29 patients (88%) showed splenomegaly. In the majority, laboratory findings were anemia (71%), neutropenia (83%), monocytopenia (85%) and/or thrombocytopenia (88%). Pancytopenia was detected in 43%. Hairy cells in peripheral blood examination were found in 90% as well as a normal lactate dehydrogenase (LDH; 91%, normal value 240-480 U/l [21]). *BRAF* V600E mutation has been assessed at diagnosis in 4 cases. In all cases, the mutation was present. Additional information on initial hematological parameters is provided in table 1. Regarding the distribution of the *IL28B* polymorphism in the whole cohort, 15 patients (41%) showed genotype CC and 19 patients (51 %) genotype CT or TT. In 3 cases (8%), genotyping was not successful. Finally, 3 patients (12.5%) were lost to follow-up.

The correlation between *IL28B* genotype and response to IFNα was analyzed in the patients treated with IFNα as first-line therapy. Genotyping was unsuccessful in 2 patients in this subgroup, who were subsequently excluded. The remaining cohort of 22 patients was split into 2 groups according to genotype (CC vs. CT/TT). The characteristics for these groups are summarized in table 2. The median follow-up time was 17 years (IQR 13-28). The initial dose was in general 3x3 Mio IU/week and dosing of maintenance therapy was mainly adapted as shown earlier [19].

RESULTS I: STUDY COHORT

Correlation sIL2R ratio with time to complete remission

For the evaluation of the levels of sIL2R all 37 patients of the study group were included. The required lab data were available in 21 cases. All patients showed a significantly increased sIL2R level at diagnosis, with values above 4'000 U/ml (table 1). Since absolute values of sIL2R show high inter-individual variability, we built a ratio between the pretreatment measurement and the one after 1 month (median 36d, IQR 29-43d) and 3 months (median 89d, IQR 83-107d). Relative decreases of the median sIL2R level of 79% (after 1 month) and 91% (after 3 months) were found. After 3 months of treatment, 9 patients (43%) showed a sIL2R level <1'000 U/ml. Due to the fact that CR rate is related to relapse-free survival [22], we correlated the sIL2R ratio to time to CR. It resulted a positive correlation for the time to CR after 1 month (rho=0.515, p=0.029) and 3 months (rho=0.550, p=0.018).

RESULTS II: IFN-SUBGROUP

Follow-up data

Follow-up data for the IFN-subgroup of 22 patients is shown in table 3. Complications occurring on IFNα-therapy were evaluated in accordance with CTCAE v4.0 [23]. Altogether, 15 patients (68%) did suffer from one or more complications due to therapy. The most common side effects were infections of grade I and II (7 cases) and persistent blood count changes (grade I and II, 5 cases). Except for two cases with grade III complications (infections with hospitalization), the remaining events were of grade I or II: intolerable flu-like symptoms (4 cases) and psychiatric symptoms (in particular lethargy, 3 cases). No grade IV event caused by IFNα was registered. In 4 cases, therapy was interrupted due to ≥1 of above-mentioned complications. In 3 patients relapse and in 1 patient primary resistance were the reasons for discontinuing IFNα-treatment. Eight patients were treated by a second-line therapy. This therapy was in 6 cases CDA, once Rituximab and one patient was retreated with IFNα. Eleven patients had a maintenance therapy with very low doses of IFNα, which was in the majority (7 patients, 63%)

3 Mio IU per one or two weeks.

Genotype and outcome

In order to examine the impact of genotype on the outcome, 2 different statistical analyses were conducted. At first, differences in the outcome of the 2 groups of genotype were explored using

Mann-Whitney test. It resulted neither significant differences between the genotypes for time on treatment (p=0.872) and time to CR (p=1.000) nor for sIL2R ratio after 1 month (p=0.343) and 3 months (p=0.530). Secondly, the association between genotype and outcome was examined using linear regression. Evaluating adjusted R^2, genotype explained 3.7% of variability of sIL2R ratio after 1 month (R^2=0.037) and 0.5% of time to CR (R^2=0.005). The variability of therapy time (R^2=-0.048) and sIL2R ratio after 3 months (R^2=-0.094) was not explained by genotype. Altogether, adjusted R^2 of these analyses complied with no or little effect size. The results are not statistical significant.

RESULTS III: SURVIVAL AND SECONDARY MALIGNANCIES

During the period of follow-up (median 14y), there was 1 death registered. The cause of death was a septic shock in aplasia, which was induced by third line therapy with Rituximab/Bendamustin at the age of 71 years, 31 years after initial diagnosis of HCL.

Regarding late effects, 6 patients (16%) suffered from secondary malignancies (table 4). Splenectomy was performed in 50% of these patients, mainly in in the early period of our observation time. Three neoplasias occurred while on IFNα therapy, 2 patients had IFNα and PNA therapy and one patient had only PNA therapy. 3 patients developed skin cancer (2 non-melanoma types), whereas the remaining had cancer of the prostate in two cases and breast cancer in one case. In average, secondary tumors were diagnosed 8 years after diagnosis of HCL (median, IQR 6-16y).

DISCUSSION

This retrospective study presents data of 37 HCL patients with a very long median follow-up time of 14 years. The IFNα treated subgroup was even observed for a median time of 17 years.

The detailed analyses of our patients show interesting findings. Whereas infections as first symptom are well known, the correlation between spleen enlargement and absent abdominal symptom is somehow surprising. However, this finding can be explained by the rather slightly enlarged spleen size (median 17 cm) and the relatively young age at diagnosis. The neutropenia and specifically the missing monocytes are a typical finding in HCL patients [5, 24, 25]. As HCL cells proliferate only slowly, LDH value would be estimated to be almost always in the normal range. In contrast to Bouafia et al., who found elevated levels in HCL patients [26], we found the expected normal LDH levels in the great majority of our patients. Therefore, LDH does not seem to be a valuable diagnostic or even prognostic parameter in HCL patients.

Despite the excellent initial response of HCL to PNA, at least 30-40% of the patients will relapse after 5 to 10 years [4]. Until now, there is no early predictive parameter to identify patients at risk. Unlimited follow-up is needed in all patients including repeated bone marrow examinations with its known limits in regards to sampling error and limit of detection [27]. SIL2R is therefore a less invasive and well-established parameter to assess disease activity in peripheral blood samples [18]. This is especially true in patients treated with IFNα, because of its unlimited treatment time and the possibility to taper down the dose in relation to sIL2R values. However, absolute values are not directly linked to the amount of HCL cells and not comparable between patients. They are therefore of no use as predictive markers. For that reason, we built a ratio between the pretreatment value and the measurement after 1 and 3 months. A correlation between the ratio and achieving a CR was calculated, because almost all patients achieved a CR (91%). However, the ratio at 1 and 3 months treatment time is significantly associated to the time to CR. Therefore, sIL2R can be used as an early marker for response. Independent of the choice of first-line therapy (IFNα or CDA), in three quarter of cases a relative drop of the sIL2R ratio of at least 50% after 1 month of therapy start was observed. This led to CR within 3.9m (median, IQR 2.5-8.9m). Equal results could be seen after 3 months with a drop of at least 80%..Regarding our results, a relative decrease of the sIL2R ratio of at least 50% after one and/or a relative decrease of 80% after 3 months can be regarded as predictor for CR within the first 8 months of treatment. Therefore, a periodically assessment of sIL2R is advisable.

In addition to the well-known sIl2R, we were looking for an association between *IL28B* genotype and treatment response in HCL patients on IFNα therapy. Because of the high effectivity of IFNα in almost all HCL patients, one could assume a direct link of the CC genotype and the disease itself. However, the genotype distribution in

our cohort was coherent with the current published literature [28, 29] in HCV patients and therefore excludes a direct link of the CC genotype and HCL. The second hypothesis of a faster, deeper or longer lasting response of the patients with a CC genotype treated with IFNα could not be proven. Explaining factors for our findings are the small sample size, confounding factors like treatment tolerance and follow-up time. The last point is of critical importance in all HCL studies, because of the excellent prognosis in these patients.
IFNα showed significantly less early side effects in the only randomized trial comparing Pentostatin and IFNα and showed less early deaths [9]. The question of secondary malignancies, one of the main long-term side effects is still an open one in HCL patients and needs to be closely looked at. The largest study about this question from Hisada et al. found an increase in secondary malignancies analysing more than 3000 HCL patients. The increase was statistically significant in patients treated with chemotherapy and in the group diagnosed between 1990 and 2002 including mainly patients treated with PNA [30]. Federico et al. included more than 1000 mainly IFNα treated patients and could not find such a difference in the estimated rate of secondary cancers [31]. Our study with a remarkable long median follow-up time of 14 years (median) showed a rate of 16% of patients with secondary malignancies, which is in line with the results of Federico et al. and others [22]. Due to the small sample size and the non-randomized study design, no additional conclusion in regards to late toxicity can be drawn. However, patients need to be informed about the risk for secondary malignancies and physicians should be vigilant for clinical signs of lymphoid, but as well solid cancers as found in our cohort and long follow-up times are essential to further investigate incidence of secondary cancers in HCL patients.

The limitations of our study are the small patient number and the retrospective design. However, the influence of *IL28B* genotype on IFNα treatment concepts was only recently recognized and prospective analysis with sufficient follow-up time is not realistic. In addition, the size of the study population needs to be viewed in the context of the rarity of HCL and the special treatment concept.

In conclusion, this study proofs that IFNα is still a valid treatment option in HCL, despite the fact that guidelines are reluctant to recommend it [6]. Whereas in the treatment of myeloproliferative neoplasm IFNα is increasingly used [32], the recognition in HCL is different. Tolerability remains one of the main argument against the use of IFNα [33], despite significantly less side effects and a lower mortality rate found in the only fully published, randomized study comparing IFNα and PNA [9]. Our study shows a good long-term tolerability with an excellent disease control. By using our sIL2R ratio, patients responding well to the treatment can be recognized early in the treatment. However, *IL28B* genotype has no influence on IFNα treatment response.

AUTHORSHIP CONTRIBUTIONS

SJ, RB and JSG designed the study, acquisitated and analyzed data and wrote the paper. KS performed blood analysis. OS reviewed the data and supported statistical analysis and interpretation. All authors critically reviewed the artical and gave final approval.

DISCLOSURE OF CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

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TABLES

Table 1: Characteristics study cohort

	First line therapy		
	Interferon-α		
	Yes	**No**	**Overall**
Number of patients (percent)	24 (65)	13 (35)	37 (100)
Age at diagnosis years - median (IQR)	46 (39-52)	45 (40-66)	45 (40-52)
Sex - number (percent)			
Male	19 (79)	10 (77)	29 (78)
Female	5 (21)	3 (23)	8 (22)
Follow-up time years - median (IQR)	17 (12-28)	6 (2-13)	14 (7-23)
Genotype - number (percent)			
Genotype CC	10 (42)	5 (38)	15 (41)
Genotype CT/TT	12 (50)	7 (54)	19 (51)
Genotyping not successful	2 (8)	1 (8)	3 (8)
Pretreatment splenectomy - number (percent)	14 (58)	4 (31)	18 (49)
Complete remission achieved - number (percent)	20 (83)	11 (84)	31 (84)
Relapse - number (percent)	5 (21)	6 (46)	11 (30)
Resistance - number (percent)	1 (4)	0	1 (3)
Hematological parameters - median (IQR)			
Hemoglobin g/l			107 (73-122)
Neutrophil granulocytes G/l			0.5 (0.3-1.2)
Monocytes G/l			0.0 (0.0-0.3)
Platelets G/l			75 (46-129)
Hairy cells in peripheral blood - number (percent)			27 (90)
LDH U/l - median (IQR)			314 (267-359)
sIL2R U/ml - median (IQR)			27'768 (16'984-540'000)
Spleen size cm - median (IQR)			17 (13-21)

Table 2: Characteristics IFN-subgroup

	Genotype	
	CC	**CT/TT**
Number of patients (percent)	10 (42)	12 (50)
Age at diagnosis years - median (IQR)	46 (36-54)	45 (39-53)
Sex - number (percent)		
Male	9 (90)	9 (75)
Female	1 (10)	3 (25)
Follow-up time years - median (IQR)	20 (11-33)	16 (14-26)
Pretreatment splenectomy - number (percent)	7 (70)	6 (50)

Table 3: Follow-up IFN-subgroup

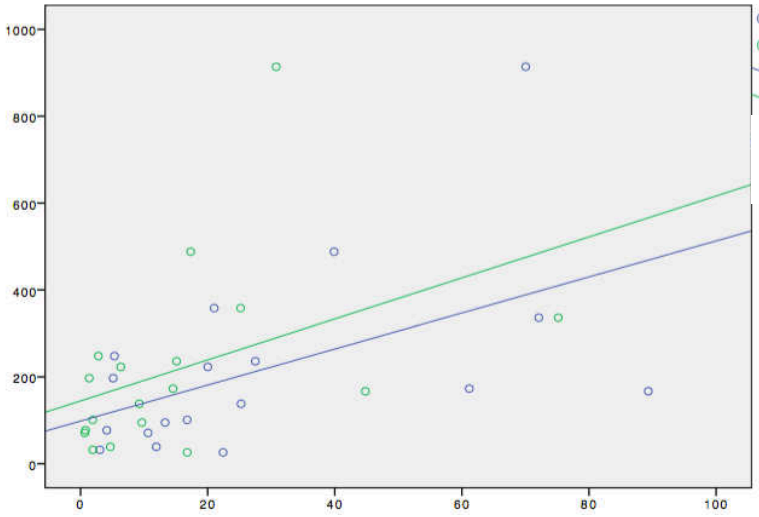
	Genotype		Significance
	CC	CT/TT	
Number of patients (percent)	10 (45)	12 (55)	
Complete remission achieved - number (percent)	10 (100)	10 (83)	0.481
Relapse - number (percent)	2 (20)	3 (25)	1.000
Resistance - number (percent)	0 (0)	1 (8)	1.000
Amount of complications under therapy	7	8	1.000
Maintenance therapy - number of patients (percent)	5 (50)	6 (50)	1.000
Discontinuation of therapy - number (percent)	5 (50)	6 (50)	1.000
Second line therapy initiated - number (percent)	3 (30)	5 (42)	0.675

Table 4: Secondary malignancies

Patient	Second cancer	Age at diagnosis of 2° cancer (y)	Therapies (1st line, 2nd line)	3rd line therapy	Time after diagnosis (y)	Time after IFN (y)	Time after PNA (y)
1	Melanoma (T1)	41	Splenectomy IFN	-	5	5	-
2	Mamma-Ca	52	IFN	CDA	13	13	9
3	Spinalioma	73	CDA	CDA	7	-	4
4	Basalioma	67	Splenectomy IFN	-	6	3	-
5	Prostate-Ca (T3)	75	Splenectomy IFN	Pentostatin	25	23	22
6	Prostate-Ca (T1)	75	IFN	-	8	8	-

VISUAL ABSTRACT

Negative correlation of relative decrease in sIL2-level to time to response



% decrease of sIL2-level after 30 d of therapy
% decrease of sIL2-level after 90 d of therapy
linear % decrease of sIL2-level after 30d/time to response
linear % decrease of sIL2-level after 90d/time to response

x: sIL-2 level in % (100% comply with level before therapy)
y: number of days to therapeutic response